P444/0001
GSL/GOSO/gl

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Inventor(s): HUANG, Dong; QI, Dong Feng

Title: NOVEL AGLYCON DAMMARANE SAPOGENINS, THEIR USE AS ANTICANCER AGENTS, AND A PROCESS FOR PRODUCING SAME

SERIAL No.: 09/910887

Filed: 24 July 2001

Examiner: Qazi, Sabiha Naim Art Unit: 1616

Date: March 4, 2005

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir,

AFFIDAVIT UNDER RULE 1.132

I, Dong Huang, of 16788 102 Avenue, Surrey, British Columbia, Canada V4N 4X2, MAKE OATH AND SAY AS FOLLOWS:

1. I have personal knowledge of the matters sworn to herein, except where the matters are stated to be based on information and belief, in which case I believe them to be true.

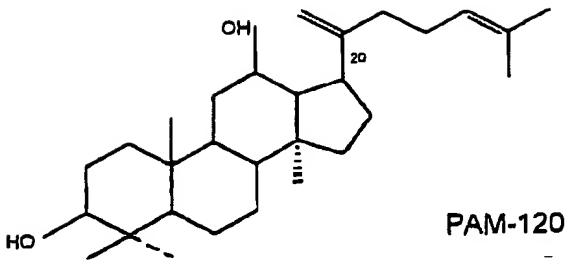
2. I am a co-inventor of the invention described and claimed in US Patent Application Serial No. 09/910887.

3. I hold a Bachelor of Science degree from the University of Beijing China.

4. I have over 20 years of experience in the fields of botany chemistry research and ginsenoside drug development.

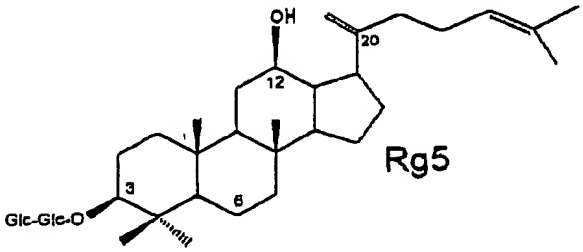
5. I have conducted side-by-side experiments to compare the efficacy of the compounds PAM-120 and Rg5.

6. PAM-120 has the following chemical structure:



PAM-120

Rg5 has the following chemical structure:



Rg5

7. I conducted experiments to compare the efficacy of PAM-120 and Rg5 against lung cancer cells in the following manner. Compounds PAM-120 and Rg5 were obtained from Pegasus Pharmaceuticals Group Inc. Human non-small-cell H460 lung cancer cells were seeded at $3x10^4$ cells/well in a 98-well plate in DMEM medium supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO_2. The cells were treated with isolated ginsenoside PAM-120 and Rg5 at a fixed dose of 25 uM. The cytotoxic effects of the compounds on the lung cancer cells were measured by determining the viability of the cells. Cell viability was measured using the MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) assay method (Denizot and Kang, J. Immunol. Meth. 89:271-277 (1986); Carmichael et al., Cancer Res. 47:936-942 (1987)) 24 hours following treatment. Cell viability was measured by determining the absorbency of stained cells. Non-viable cells have lower absorbency compared to viable cells. Table 1 shows the viability of H460 lung cancer cells in the presence of the compound PAM-120 and Rg5 at 25 uM.

Compound (25 uM)	Absorbency of stained cells (M±SD)	Viability (%)
Blank Control	0.352±0.062	100.00
PAM-120	0.218±0.043	61.93
Rg5	0.290±0.065	82.38

Table 1: Viability of H460 Lung Cancer Cells in the Presence of 25 uM Pam-120 and Rg5.

8. The results in Table 1 illustrate that H460 lung cancer cells are significantly less viable in the presence of PAM-120 than Rg5. Therefore PAM-120 has greater cytotoxic effects than Rg5.

9. I conducted experiments to compare the efficacy of PAM-120 and Rg5 against drug-resistant breast cancer cells in the following manner. Compounds PAM-120 and Rg5 were obtained from Pegasus Pharmaceuticals Group Inc. Human drug-resistant MCF7r breast cancer ells were seeded at $3x10^4$ ells/well in a 98-well plate in DMEM medium supplemented with 10% fetal bovine serum and incubated at 37℃ with 5% CO_2. The cells were treated with isolated ginsenoside PAM-120 and Rg5 at various concentrations. The IC50s of the compound PAM-120 and Rg3 were determined using standard methods. IC50 is the concentration of a compound needed to reduce the growth of a population of cells by 50%. The IC50s of the compounds are shown in Table 2.

Compound	IC50 (μg/mL)
PAM-120	<10
Rg5	70 ± 5.4

Table 2: IC50 Values of Compounds PAM-120 and Rg5 against MCF7r Breast Cancer Cells

10. The results in Table 2 illustrate that PAM-120 has a significantly lower IC50 than Rg5. PAM-120 has an IC50 over 7 times less than the IC50 of Rg5. Therefore, PAM-120 is effective at inhibiting MCF7r breast cancer cells at a much lower concentration than Rg5.

11. I conducted experiments to compare the efficacy of PAM-120 and Rg5 against melanoma cells in the following manner. Compounds PAM-120 and Rg5 were obtained from Pegasus Pharmaceuticals Group Inc. Mouse B16 melanoma ells were seeded at $3x10^4$ ells/well in a 98-well plate in DMEM medium supplemented with 10% fetal bovine serum and incubated at 37℃ with 5% CO_2. The cells were treated with isolated ginsenoside PAM-120 and Rg5 at various concentrations. The IC50s of the compound PAM-120 and Rg5 were determined using standard methods. IC50 is the concentration of a compound needed to reduce the growth of a population of cells by 50%. The IC50s of the compounds are shown in Table 3.

Compound	IC50 (μg/mL)
PAM-120	<10
Rg5	35 ± 3.9

Table 3: IC50 Values of Compounds PAM-120 and Rg5 against B16 melanoma Cells

12. The results in Table 3 illustrate that PAM-120 has a significantly lower IC50 than Rg5. PAM-120 has an IC50 value over 3 times less than the IC50 of Rg5. Therefore, PAM-120 is effective at inhibiting B16 melanoma cells at a significantly lower concentration than Rg5.

SWORN before me at the city of)
SURREY, in the province of British)
Columbia, Canada this 7 day of March)
2005)
)
_____)
A Notary Public in and for the)
Province of British)
Columbia, Canada. My Commission is for life)

(Dong Huang)

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